# Chronic course of a hemolytic uremic syndrome caused by a deficiency of factor H-related proteins (CFHR1 and CFHR3)

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## CASE PRESENTATION

**A 36-year-old patient complained of progressing fatigue, lack of appetite, and weakness for a few weeks, for which he had been using paracetamol (acetaminophen) intermittently. He was referred to our center from another hospital with hemolysis, thrombocytopenia, and acute renal failure (ARF). On admission, the patient did not complain of any specific additional symptoms. Besides paracetamol, he had not received any other medication. The patient reported flu-like symptoms 3 months before admission. The family history was unremarkable. Physical examination revealed a pale-looking patient (180 cm; 81 kg) with icteric sclerae. He was tachycardic (110 heart beats per min) and had elevated blood pressure (155/90 mm Hg). No other physical abnormalities were detectable.**

**Laboratory investigations are depicted in Table 1. Specific analyses: von Willebrand factor cleavage protease activity 31% (40–120%), von Willebrand Factor Multimere negative, antibodies to von Willebrand Factor cleavage protease negative, factor H 614 mg $l^{-1}$ (345–590 mg $l^{-1}$). Western blot analyses with patient's serum revealed the presence of complement factor H (CFH) and complement factor H-like protein 1 (CFHL1), but no detectable levels of complement factor H-related proteins 1 and 3 (CFHR1 and CFHR3) (Figure 1a). Antibodies to CFHR1 were negative. Genetic analyses[1] showed no *CFH* mutation, but revealed homozygous deletion of a 83 kb genomic fragment representing *CFHR3* and *CFHR1* (Figure 1b). Kidneys were of normal size with increased density by ultrasound examination. Electrocardiography revealed ischemic changes posteroseptally, and hypertrophy of the left ventricle was diagnosed by echocardiography.**

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## RENAL BIOPSY

Renal biopsy contained 32 glomeruli. Several glomerular segments and preglomerular arterioles were occluded by hyaline thrombi as verified by immunohistology (Figure 2a and b). By electron microscopy (Figure 2c and d), glomeruli showed segmental detachment of endothelial cells from the basement membrane, with loss of fenestrae. Mesangial edema and segmental mesangial matrix increase could be observed. The tubulointerstitial compartment was characterized by focal interstitial fibrosis with collapsed tubules with broadened basement membranes. Mononuclear cells were irregularly distributed in these areas; interstitial fibrosis and atrophy were estimated at 30–40%. By immunohistology applying the alkaline phosphatase anti-alkaline phosphatase (APAAP) method, a strong signal for fibrinogen/fibrin could be seen in thrombi and along glomerular capillaries (Figure 2b).

## CLINICAL DIAGNOSIS AND DIFFERENTIAL DIAGNOSES

Atypical D(−) hemolytic uremic syndrome (HUS) with ARF, chronic interstitial fibrosis, and myocardial ischemia.

The triad of ARF, hemolytic anemia, and thrombocytopenia may be seen in severe sepsis, disseminated intravascular coagulation, rheumatic systemic disorders, heparin-induced thrombocytopenia, malignant hypertension, and thrombotic-thrombocytopenic purpura (TTP).[1] The first three diagnoses could be excluded because of clinical history and clinical chemistry analyses. Heparin-induced thrombocytopenia could be excluded owing to non-exposure. Because of the high shear forces generated by high blood pressure, schistocytes may form in malignant hypertension, but not in large numbers, as they are to be found in thrombotic microangiopathies.[1] Severe deficiency of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) has been identified mostly in those cases described clinically as TTP. However, distinction of TTP and HUS based on ADAMTS13 activity is not without criticism,[2] and should rather be based on clinical manifestations.[3] Moreover, results from ADAMTS13 activity measurement are often time-delayed and therapeutic decisions should thereof be made independently.

**Table 1 | Relevant laboratory values**

| | Patient | Normal range |
|---|---|---|
| Hemoglobin | 5.0 g per 100 ml | 13.5–17.5 g per 100 ml |
| Platelets | 88.8 $\times 10^3/\mu l$ | 150–350 $\times 10^3/\mu l$ |
| Schistocytes (blood smear) | 45‰ | $\leqslant$5‰ |
| Reticulocytes | 108‰ | $\leqslant$20‰ |
| Free hemoglobin | 33.1 mg per 100 ml | $\leqslant$10 mg per 100 ml |
| Creatinine | 6.7 mg per 100 ml | 0.6–1.1 mg per 100 ml |
| BUN | 78 mg per 100 ml | 8–21 mg per 100 ml |
| Creatinine clearance | 15 ml $min^{-1}$ | 80–160 ml $min^{-1}$ |
| Phosphorous | 1.77 mmol $l^{-1}$ | 0.7–1.4 mmol $l^{-1}$ |
| Uric acid | 14.1 mg per 100 ml | 3.5–7.4 mg per 100 ml |
| Bilirubin | 2.1 mg per 100 ml | $\leqslant$1.2 mg per 100 ml |
| Glutamat-oxalacetat-transminase | 67 U $l^{-1}$ | $<$25 U $l^{-1}$ |
| Lactate dehydrogenase | 1824 U $l^{-1}$ | $\leqslant$232 U $l^{-1}$ |
| Haptoglobin | $<$0.1 g $l^{-1}$ | 0.45–2.05 g $l^{-1}$ |
| Creatine kinase | 362 U $l^{-1}$ | $\leqslant$270 U $l^{-1}$ |
| Creatine kinase (myocard type) | 24 U $l^{-1}$ | $\leqslant$17 U $l^{-1}$ |
| Complement C3c | 0.49 g $l^{-1}$ | 0.9–1.8 g $l^{-1}$ |
| Urinalysis | Blood +++, protein +++ | Blood −, protein − |
| Urine sediment examination (per visual field) | $>$20 erythrocytes ($<$5% acanthocytes), 0–1 erythrocyte casts, 0–1 hyalinic casts | 1–4 erythrocytes, 0 erythrocyte casts, 0 hyalinic casts |
| Proteinuria | 3950 mg $day^{-1}$ (albumine 1010 mg $l^{-1}$, $\alpha$1-microglobuline 59 mg $l^{-1}$, IgG 93.1 mg $l^{-1}$) | $<$200 mg $day^{-1}$ |

BUN, blood urea nitrogen.
The other analyses did not show pathological changes.

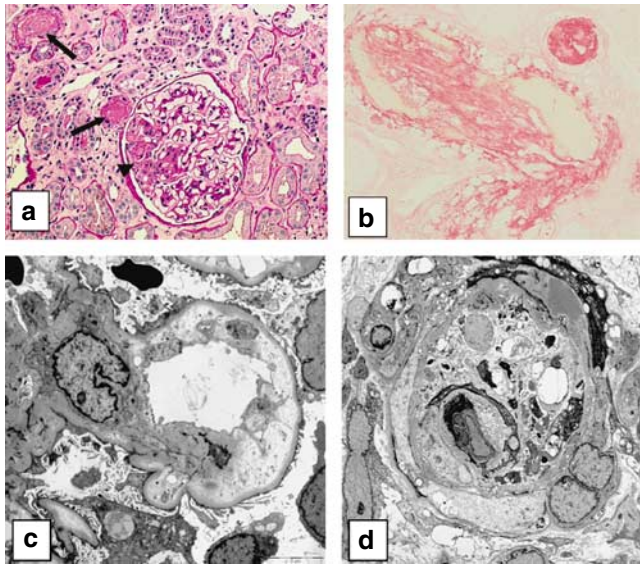


**Figure 2 | Histological changes seen in renal biopsy.** (**a**) Glomerulus with occlusion of capillaries by hyaline thrombi in 25% of capillary convolute (arrowhead). Two transverse sections of afferent arteriole show a complete obliteration by hyaline thrombus (arrows). There is focal interstitial fibrosis surrounding tubules with broadened basement membranes (PAS staining, original magnification $\times$200). (**b**) Tangential and transverse section of arterioles with thrombus, positive for fibrinogen/fibrin (APAAP, original magnification $\times$400). (**c**) Transmission electron micrograph of glomerulus, endothelium is segmentally detached from glomerular basement with electrolucent subendothelial space. Fenestrae are lost. Foot processes of podocytes are often plumb and only rarely effaced. (TEM, original magnification $\times$3000; Zeiss EM 900). (**d**) Arteriole with occlusion of lumen by debris and fibrin, endothelium is necrotic as are some smooth muscle cells. (TEM, original magnification $\times$3000; Zeiss EM 900 (Jena, Germany)).

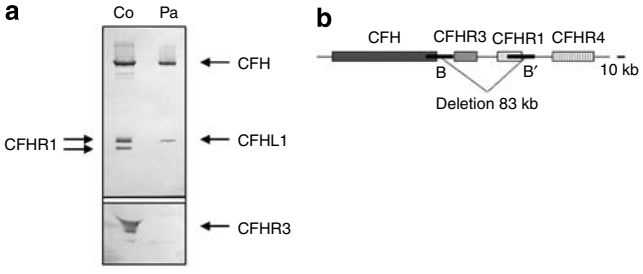


**Figure 1 | Western blot analysis for complement factor H-related protein 1 (CFHR1) and CFHR3 and deletion of a chromosomal 83 kb fragment in DNA derived from the patient.** (**a**) Plasma (Pa) of the patient was separated by SDS-PAGE, and analyzed by western blotting using an antiserum, that identifies factor H (150 kDa) and CFHR1 (42 and 37 kDa) (lane 2) or CFHR3 (55 kDa) (inset). In the patient's (Pa), the two CFHR1 forms and CFHR3 are absent. (**b**) Both alleles of the patients include all the exons of the *CFH* and *CFHR4* genes, but show deletion of the *CFHR3* and *CFHR1* genes. Analyses of the genetic deletion were performed as described.[17]

## CLINICAL FOLLOW-UP

Plasma exchange (PE) using fresh frozen plasma was initiated with 3000 ml of plasma per session ($\sim$40 ml plasma per kg body weight). In addition, 1 mg per kg body weight prednisolone was given. After transfusion of two units of erythrocyte concentrates, Hb increased to 9.0 g per 100 ml. However, ischemia typical ECG changes did not disappear until the fourth PE (Hb 9.1 g per 100 ml). Daily PE was continued until day 23 and then stepwise reduced over the subsequent 10 weeks. The blood pressure was controlled initially (first 8 weeks) with metoprolol and moxonidin, thereafter with metoprolol and benazepril. The left ventricular hypertrophy suggested a so far unrecognized arterial hypertension possibly caused by subclinical kidney involvement of the underlying disease. However, this is speculative.

Under this therapy, creatinine clearance improved to 54.4 ml $min^{-1}$ and serum creatinine of 2.3 mg per 100 ml, respectively. Although renal function stabilized, persistent proteinuria was noted with 2520 mg $day^{-1}$ after 14 weeks. Thus, a dual blockade of the renin–angiotensin system with benazepril and valsartan in addition to metoprolol and amlodipin was administered with blood pressure controlled to mean values about 120/80 mm Hg, whereas moxonidin was withdrawn. Subsequently, the patient developed two relapses with increased hemolytic activity, platelet and

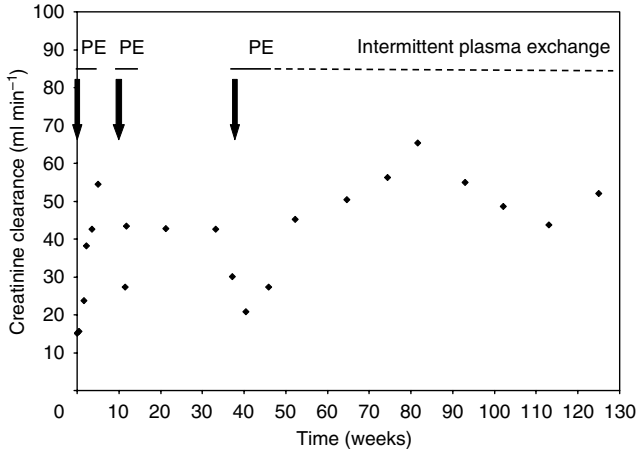


**Figure 3 | Time course of creatinine clearance and PE therapy.** There is an initial improvement with subsequent stabilization of renal function under intermittent PE during the last 19 months. Relapse of HUS (arrow); daily PE (drawn line); intermittent plasma exchange (dotted line).

complement component C3c decreases, and further deterioration of renal excretory function within 6 months after his first discharge from our hospital. These relapses were again treated with additional cycles of daily PE, followed each time by reductions of disease activity. However, a slight hemolytic activity (elevated lactate dehydrogenase, decreased haptoglobin levels, and presence of schistocytes) persisted and renal function deteriorated to a creatinine clearance between 27 and 42 ml $min^{-1}$. In view of persistent hemolysis, twice-weekly maintenance PE was prescribed. With this regimen, lactate dehydrogenase was almost normal (range 235–285 U $l^{-1}$) and platelets were within normal range, although haptoglobin remained decreased below 0.1 g $l^{-1}$. Attempts of reducing the frequency of PE resulted in immediate relapses. Thus, maintenance PE has been continued for a period of 19 months as of the date of this report resulting in a stabilization of renal function (current creatinine clearance 51 ml $min^{-1}$) (Figure 3).

## DISCUSSION

### History, epidemiology, and classification

In 1955, Gasser[4] coined the term 'hemolytic uremic syndrome.' HUS is characterized by thrombocytopenia, hemolytic anemia, and low platelet count. Commonly accepted parameters and terms for describing different forms of HUS are still lacking. It is now accepted to classify HUS based on the presence or absence of prodromal diarrhea. HUS with prodromal diarrhea is called typical HUS (also called epidemic or D(+)HUS). HUS without prodromal diarrhea is called atypical HUS (also called sporadic or D(−)HUS).[1,5] The idiopathic (atypical) D(−)HUS is the most common form of microangiopathy with renal failure in adults.[1] D(−)HUS is rare, may be familial, and has a poorer prognosis, with death rates of up to 25% in the acute phase and 50% requiring ongoing renal replacement therapy.[6]

### Clinical manifestation of HUS

ARF may vary from simple hematuria, proteinuria to severe oliguria. ARF with anuria has been reported in HUS in 40% of patients, and up to 61% have to undergo dialysis. Other systems affected include central nervous system, gastrointestinal tract, liver, and pancreas.[7] Fever and neurological dysfunction are more commonly found in patients with TTP but have been described in HUS as well. Similarly, the presence of pathologic lesions in pancreas, brain, adrenal glands, and heart was significantly more common in TTP, but can also be found in HUS.[8]

### Pathology

In D(−)HUS, the predominant pathological abnormality is found in the renal arterioles and glomeruli. There is widespread endothelial swelling with cell retraction leading to exposure of the basement membrane. The vessel lumens are occluded by platelet fibrin thrombi.[6,9] Interestingly, plasma of patients with D(−)HUS, but not with D(+)HUS induced apoptosis of endothelial cells and expression of Fas in microvascular endothelial cells of renal origin.[1,10]

### Pathogenesis and diagnosis of HUS

D(−)HUS is characterized by activation of the complement cascade via the alternative pathway. Classically, laboratory investigations reveal a decrease of complement component C3 but normal C4, as it was the case in our patient.[11] The alternative pathway of complement represents a safeguard system in humans, which is initiated in the fluid phase by the spontaneous generation of an enzyme that cleaves C3, that is by the first C3 convertase. The activated complement system has devastating effects, therefore important control and inhibitory reactions exist on host cells, which keep the system on track and provide local protection of host surfaces and cells. This local control ensures that activation is specifically targeted to foreign surfaces. Defective complement control is linked to HUS.[12] Formation and activity of the alternative pathway amplification convertase C3bBb is tightly controlled both in the fluid phase and on the surface of host cells or tissues (Figure 4). Four potent regulators are expressed on the surface of host cells, that is, CR1 (CD35), MCP (membrane cofactor protein, CD46), DAF (decay accelerating factor, CD55) and CD59.[11,13] Potent regulators are also found in the fluid phase consisting of the members of the factor H protein family. This family of structurally and immunologically related proteins includes factor H (CFH), factor H-like protein 1 (CFHL1), and six complement factor H-related proteins (CFHR) denoted CFHR1, CFHR2, CFHR3, CFHR4A, CFHR4B, and CFHR5.[14,15,16] The fluid phase regulator CFH is an important and potent inhibitor of the amplification cascade of complement activation. It is estimated that CFH in combination with factor I controls more than 99% of complement activity.[13] All these proteins are synthesized primarily in the liver and are secreted into plasma.[16] The functional properties of CFHL1 and CFHR proteins are not fully defined. CFHL1 shows the complement

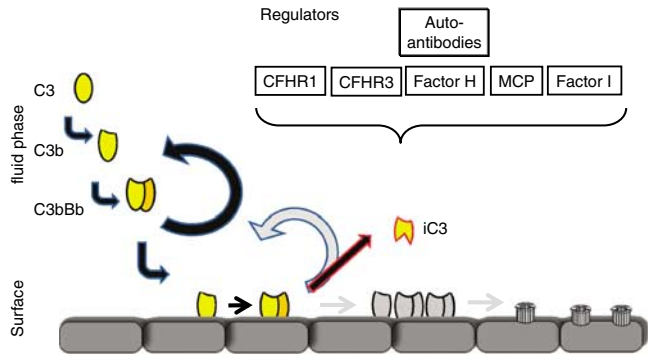


**Figure 4 | Activation steps of the alternative pathway of complement.** The alternative pathway of complement is initiated spontaneously by a conformational change of the C3 protein. After binding of factor B and conversion to Bb, the resulting C3Bb fragment forms an active complement convertase that generates more C3b. At the surface, C3b is generated, which deposits onto the surface. In the absence of regulator's activation proceeds, a powerful amplification reaction is generated and results in the formation of membrane attack complexes (MAC), which generates pores and causes cell damage. On non-activator host cells, formation of MAC complexes is prevented by the action of multiple regulators, like the fluid phase regulators CFHR1, CFHR3, and factor H, and membrane bound regulators (MCP/CD46), the serine protease factor I which in the presence of a cofactor results in the inactivation of C3b and the formation of inactive form of C3b, i.e., iC3b. Apparently, the components (C3 and Factor B) as well as these regulators are risk factors for HUS as mutations in these genes or chromosomal deletion are associated with these diseases.

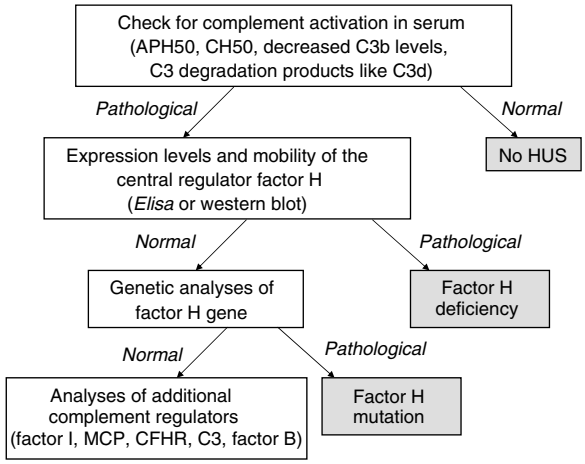


**Figure 5 | Diagnostic algorithm of atypical HUS according to Zipfel and Skerka.**[12] As diagnosis of atypical HUS can be rather expensive, the rationale for analysis is presented as a hierarchical order according to a diagnostic algorithm which was recently proposed.[13]

regulatory properties homologous to CFH.[13] A possible diagnostic algorithm of atypical HUS is shown in Figure 5. None of the identified disease-associated complement genes, however, can be considered as the initial trigger, which initiates reactions that cause tissue damage and atypical HUS. Thus, the initial trigger of HUS remains a matter of speculation: it may be an infectious agent or an inflammatory stimulus that activates complement locally.[12] These trigger mechanisms include infection with neuraminidase-producing *Streptococcus pneumoniae* or HIV, pregnancy, rheumatological disorder, malignancy, combined methylmalonic aciduria and homocystinuria, a previous type of transplants, especially hematopoietic cell transplantation as well as the treatment with several drugs.[9] However, all this could be excluded in our patient.

### CFHR in HUS

To date, similarities have been observed in the ability of CFHRs to bind C3b in homology to CFH.[14] In our patient, we found a deficiency of CFHR1 and CFHR3. Deficiency of CFHR1 and CFHR3 was identified as risk factors for the appearance of atypical HUS in one study. In cohort analyses, the absence of CFHR1 and CFHR3 has been identified in 16–28% in patients with atypical HUS identifying a homozygous and heterozygous genomic deletion as possible causes.[17] The functional relevance has been shown in three CFHR1/CFHR3-deficient patients from whom heat-inactivated serum increased erythrocyte lysis. These data showed that CFHR1/CFHR3-deficient plasma reduce protective activity and suggested that the absence of CFHR1 and/or CFHR3 contributes to the defective regulation of complement activation on cell and tissue surfaces.[17]

### Treatment of CFHR deficiency-related HUS

The time course of patients with CFHR1/CFHR3 deficiency has been described in only three individuals at the age of 11–13 years, whose disease activity could be controlled by PE, intravenous plasma infusion, corticosteroids, and antihypertensives for 1–4 years.[17,18] Our case is the first description of an adult patient with a CFHR1/CFHR3 deficiency who is followed over an extended period of 19 months. Despite PE twice a week, laboratory investigations revealed a slight continuous disease activity without an apparent further damage in the kidney as assessed by stable renal function parameters. In a previous study, it has been speculated how this deficiency exerts disease-modifying action. One explanation was that CFHR1 and CFHR3 have cofactor-enhancing activity for CFH. On the other hand, a regulatory function in C3b processing was discussed, as CFHR3 binds C3b and heparin.[19] Another possibility was that the deletion is in linkage disequilibrium with other susceptibility alleles in CFH or that it may affect CFH transcription.[17,19]

## CONCLUSION

The time course of chronic atypical HUS due to a deficiency of CFHR1 and CFHR3 can be positively influenced by regular PE, resulting in disease remission over a long period and stabilization of renal function. However, the long-term prognosis remains unclear.

### ACKNOWLEDGMENTS

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